

Atty Dkt No.: STAN-202
USSN: 09/972,031

AMENDMENTS

IN THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method of enhancing discrimination discriminating among a plurality of nucleic acid targets, the method comprising:

~~enhancing differences in the extent of nucleic-acid-duplex formation between each of said nucleic-acid-targets and at least one common nucleic-acid probe by forming nucleic acid duplexes between said nucleic acid targets and said at least one common nucleic acid probe in a hybridization reaction performed in the presence of a specific association enhancer under conditions suitable for accelerated association of specific duplexes having a perfectly matched region of complementarity,~~

~~whereby differences in the extent of specific duplex formation discriminate among said nucleic-acid-targets between matched and mismatched duplex regions at the level of a single nucleotide difference.~~

2. (Original) The method of claim 1, wherein said specific association enhancer is a cationic detergent.

3. (Original) The method of claim 2, wherein said cationic detergent is selected from the group consisting of tetradecyltrimethylammonium salts, cetyltrimethylammonium salts, and octadecyltrimethylammonium salts.

4. (Original) The method of claim 3, wherein said cationic detergent is selected from the group consisting of cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), cetyltrimethylammonium hydroxide (CTAS), tetradearyltrimethylammonium bromide (TTAB), and octadecyltrimethylammonium bromide (OTAB).

5. (Currently Amended) The method of claim [14] 2, wherein said cationic detergent is cetyltrimethylammonium bromide.

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6. (Previously Presented) The method of claim 1, wherein each of said formed duplexes includes a molecule of RNA and a molecule of DNA.

7. (Previously Presented) The method of claim 1, wherein said each of said formed duplexes includes two molecules of RNA.

8. (Previously Presented) The method of claim 1, wherein each of said formed duplexes includes a molecule of DNA and a molecule of modified DNA (mDNA).

9. (Original) The method of claim 8, wherein the mDNA molecule includes at least one nucleotide modified at the 2' carbon of ribose.

10. (Previously Presented) The method of claim 1, wherein said at least one common probe comprises a region of complementarity of at least 16 nucleotides in length to at least one of said targets.

11. (Previously Presented) The method of claim 1, wherein said at least one common probe comprises a region of complementarity of no more than 30 nucleotides in length to at least one of said targets.

12. (Previously Presented) The method of claim 1, wherein each of said formed duplexes includes a nucleic acid molecule no more than 30 nt in length.

13. (Previously Presented) The method of claim 12, wherein each of said formed duplexes includes a nucleic acid molecule at least 16 nt in length.

14. (Previously Presented) The method of claim 13, wherein each of said formed duplexes includes a nucleic acid molecule 16 - 30 nt in length.

15. (Original) The method of claim 1, wherein said plurality of targets includes at least 5 targets of distinct sequence.

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16. (Original) The method of claim 15, wherein said plurality of targets includes at least 100 targets of distinct sequence.

17. (Original) The method of claim 1, wherein said targets are genomic DNA.

18. (Original) The method of claim 1, wherein said targets are mRNA or cDNA.

19. (Original) The method of claim 1, wherein said targets are derived from mammalian nucleic acids.

20. (Original) The method of claim 19, wherein said mammalian nucleic acids are human nucleic acids.

21. (Original) The method of claim 1, wherein said at least one common probe is genomic DNA.

22. (Original) The method of claim 1, wherein said at least one common probe is mRNA or cDNA.

23. (Previously Presented) The method of claim 1, wherein said at least one common probe is derived from mammalian nucleic acids.

24. (Original) The method of claim 23, wherein said mammalian nucleic acids are human nucleic acids.

25. (Previously Presented) The method of claim 1, wherein said formed duplexes are formed in a common hybridization reaction.

26. (Previously Presented) The method of claim 1, wherein said hybridization reaction is a single phase solution reaction.

27. (Previously Presented) The method of claim 1, wherein said at least one common probe, or each of said targets, is immobilized on a substrate.

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28. (Previously Presented) The method of claim 1, wherein said at least one probe, or each of said targets, is detectably labeled.

29. (Previously Presented) The method of claim 1, wherein said hybridization reaction comprises less than about 0.7M total ionic salt concentration.

30. (Previously Presented) The method of claim 1, wherein said hybridization reaction is performed at a temperature of no more than about 60°C.

31. (Original) The method of claim 1, wherein at least two of said plurality of targets differ in sequence by no more than a single nucleotide.

32. (Previously Presented) The method of claim 1, further comprising, after duplex formation:
adding salt to said hybridization reaction until said hybridization reaction comprises greater than 0.7M total ionic salt concentration; and
removing or diluting said specific association enhancer.

33. (Previously Presented) The method of claim 1 or claim 32, further comprising:
separating said formed nucleic acid duplexes from said hybridization reaction for use in a subsequent enzymatic reaction.

34. (Withdrawn) A method of performing a hybridization-primed enzymatic reaction,
comprising:

hybridizing at least one nucleic acid primer to a nucleic acid template in the presence of an effective amount of a specific association enhancer, wherein said at least one primer has a region of complementarity to said template, and then

performing an enzymatic reaction on said duplexed primer.

35. (Withdrawn) The method of claim 34, wherein said primer is DNA and said template is RNA.

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36. (Withdrawn) The method of claim 34, wherein said primer is RNA and said template is DNA.

37. (Withdrawn) The method of claim 34, wherein said specific association enhancer is a cationic detergent.

38. (Withdrawn) The method of claim 37, wherein said cationic detergent is selected from the group consisting of tetradecyltrimethylammonium salts, cetyltrimethylammonium salts, and octadecyltrimethylammonium salts.

39. (Withdrawn) The method of claim 38, wherein said cationic detergent is selected from the group consisting of cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), cetyltrimethylammonium hydro sulfate (CTAS), tetradecyltrimethylammonium bromide (TTAB), and octadecyltrimethylammonium bromide (OTAB).

40. (Withdrawn) The method of claim 39, wherein said cationic detergent is cetyltrimethylammonium bromide.

41. (Withdrawn) The method of claim 34, wherein said enzymatic reaction is selected from the group consisting of: polymerization, nuclease digestion, phosphatasing, phosphorylation, methylation, and ligation.

42. (Withdrawn) The method of claim 41, wherein said enzymatic reaction is polymerization.

43. (Withdrawn) The method of claim 34, further comprising the step, after probe hybridization and before enzymatic reaction, of:
removing said specific association enhancer.

44. (Withdrawn) The method of claim 43, further comprising the step, before removing said specific association enhancer, of:

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adding salt to said hybridization reaction.

45. (Currently Amended) A method for increasing the specific association rate of a pair of single-stranded nucleic acid molecules, the method comprising:

combining in a reaction mixture a first single-stranded molecule and a second single-stranded molecule in the presence of a specific association enhancer, said combining being under conditions suitable for accelerated association of the first and second molecules in into a specific nucleic acid duplex having a perfectly matched region of complementarity.